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OPTIMIZATION OF METHANE PRODUCTION FROM ANAEROBIC CO-DIGESTION OF MUNICIPAL SOLID WASTE AND SEWAGE SLUDGE

Master of Science Thesis

Examiner: Professor Jukka Rintala
Examiner and topic approved by the Faculty Council of Natural Sciences on August 13, 2014.

ABSTRACT

TAMPERE UNIVERSITY OF TECHNOLOGY
Master's Degree Programme in Science and Bioengineering
FATHI AGHDAM, EHSAN: Optimization of methane production from anaerobic co-digestion of municipal solid waste and sewage sludge
Master of Science Thesis, 42 pages
October 2014
Major subject: Biotechnology
Examiner: Professor Jukka Rintala
Keywords: Anaerobic Digestion, Municipal Solid Waste, Sewage Sludge, Co-digestion

Anaerobic digestion (AD) of sieved and shredded organic fraction of municipal solid waste (OFMSW), and source-separated biowaste (BW) and sewage sludge (SS), and co-digestion of BW and SS were studied in laboratory scale semi-continuously fed continuous stirring tank reactors (CSTRs) at 35 °C with organic loading rate (OLR) from 1 to 2 kg volatile solid (VS) per m^3 d. The aim of this work was to study effect of OLR on reactor performance, comparing the effect of co-digestion of BW and SS with mono-digestion of SS, and comparison between source-separation of BW and mechanical treatment of OFMSW regarding the resulted methane yield.

Average methane yield of 386, 385, 198, and 318 L CH_4/kg VS were obtained for OFMSW, BW, SS, and co-digestion of BW and SS respectively in reactor experiment. Process was stable and there was not volatile fatty acids (VFA) accumulation and pH fluctuation. Average methane yield of SS was increased by 61% as a result of co-digestion with BW. VS removal of SS could increase from 36% to 85% as a result of co-digestion with BW.

Methane yield of 603, 534, and 369 L CH_4/kg VS were obtained for BW, OFMSW and SS respectively in batch experiments at 35 °C. Methane yield of source-separated BW was 12% higher than methane yield observed for mechanically treated OFMSW, which can be interpreted as a positive effect of source-separation on methane yield.

In conclusion, AD of OFMSW, BW, and SS and co-digestion of BW and SS were shown to be feasible with OLR of 1 to 2 kg VS/m^3 d in mesophilic conditions. OFMSW and BW were found to have higher methane yield (around 2 times) than SS. Co-digestion of BW and SS could increase methane yield and improve reactor performance in comparison to mono-digestion of SS. Source-separated BW could result in higher methane yield than mechanically treated OFMSW in batch experiment.

PREFACE

This thesis work was carried out within the Chemistry and Bioengineering Department at Tampere University of Technology during the period of February 2014 to October 2014. This project was funded by Gasum Company and Maa-ja Vesitekniikan Tuki foundation.

First of all, I would like to acknowledge my supervisor, Professor Jukka Rintala, for his kind supervision, support and guidance during my M.Sc. thesis.

I would like to acknowledge all of the people who have ever helped me during my M.Sc. thesis. Special acknowledgement is given to Viljami Kinnunen and Antti Nuottajärvi for their help with my experiment set-up and analysis done during the experiment period.

Last but not least, I would like to thank my family and friends for their support and encouragement during my M.Sc. studies.

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October 14, 2014

LIST OF ABBREVIATIONS

AD	Anaerobic digestion
BMP	Biochemical methane potential
BPR	Biogas production rate
BW	Bio-waste
CSTR	Continuous stirring tank reactor
EoW	End-of-Waste
FSWA	Finnish solid waste association
FW	Food waste
GHG	Greenhouse gas
HRT	Hydraulic retention time
LCFA	Long chain fatty acid
LFW	Leather fleshing waste
MSW	Municipal solid waste
OLR	Organic loading rate
OFMSW	Organic fraction of municipal solid waste
SBP	Specific biogas production
SS-OFMSW	Source sorted organic fraction of municipal solid waste
SCOD	Soluble chemical oxygen demand
SRB	Sulfate reducing bacteria
SS	Sewage sludge
TCD	Thermal conductivity detector
TVFA	Total volatile fatty acid
TS	Total solid
VS	Volatile solid
VSS	Volatile suspended solid
VFA	Volatile fatty acid
WWTP	Waste water treatment plant

CONTENTS

1. INTRODUCTION

Global municipal solid waste (MSW) production reached 1.3 billion tonnes per year in 2010 and it is expected to increase to 2.2 billion tonnes per year by 2025. Because the population of world is constantly increasing it leads to more activities and waste generation. The disposal of constantly increasing volume of waste in a way that is sustainable and does not harm the environment is a big challenge (Zheng et al. 2013).

Landfilling is the main method for waste disposal in many countries. In 2010, 54% of MSW went to landfill in the United States (US Environmental Protection Agency, 2010) while in 2011, 77% of the MSW in China was disposed of in landfills (China NBS, 2012). However, landfilling has lots of environmental impacts: greenhouse gas effects, affecting ozone layer, toxic volatile organic compounds, odor because of H_2S production, noise of transportation vehicles, risk of fire because of methane gas production, and soil and water pollution (Christensen 2011).

Because of these environmental impacts of landfilling, EU Directive on the landfill of waste (99/31/EC) was conducted to prevent or reduce these effects of the landfill of waste. According to it, the amount of biodegradable municipal waste should be reduced to 35% of 1995 level by 2016. So there is a need of searching for alternatives for landfilling. AD is a good alternative which also produces biogas, and provides a valuable effluent rich in nutrients that can be applied on soil as fertilizer.

SS is the sludge from urban waste water treatment plants. Because the population of world is always increasing, the amount of the generated sewer is also increasing which results in building new wastewater treatment plants (WWTPs) and production of more sludge (Guo et al. 2013). As this sludge might contain chemical and/or biological contaminants, it cannot be applied on soil before treatment or discharged in water bodies. Thus the disposal of sludge is also a big challenge and the cost may be as high as 50–60% of the total operational costs of WWTPs (Li et al. 2013).

SS is rich in nutrients (N, P and K) and can be used as fertilizer but there is a risk of the presence of chemicals (heavy metals and organic xenobiotic) and biological contaminants (enteric parasites, virus and pathogen bacteria) in the sludge (Scaglia et al. 2014). In order to ensure human health and protection of environment, sludge must be treated (European Commission, 2000).

In different countries, there are different ways of disposal and regulations. In general, landfilling and land-spreading has been the main methods of SS disposal. The major alternatives to land-spreading and landfill are incineration and AD of SS. Many countries inside EU and also USA and Japan have used incineration since long time ago, and also currently there are high investments in different countries for incineration of SS (Donatello et al. 2013). AD has been used for treatment of sludge for more than a century. AD of organic waste with high moisture content, such as SS, is promising for both energy and material recovery (Hidaka et al. 2013). Because SS has high moisture, energy recovery by incineration is poor and AD is more cost-efficient (Zupancic et al. 2008).

Many authors have done research in this field in recent years. Zupancic et al. (2008) studied the co-digestion of SS (mixture of primary and secondary sludge) and OFMSW (domestic refuse), and reported improvement in reactor performance as shown by the increased volatile suspended solid (VSS) degradation efficiency, and higher biogas production and methane yield in comparison to mono-digestion of SS. Sosnowski et al. (2003) found that co-digestion of SS (mixture of primary sludge and thickened excess activated sludge) and OFMSW (source-separated kitchen waste) can enhance AD by increase in methane yield.

The objective of this study was to evaluate the feasibility of OFMSW, BW and SS as substrates for biogas production in laboratory scale CSTRs at 35 °C. OLRs varied between 1 and 2 to study its impact on biogas production, methane yield and reactors performance. Also the influence of co-digestion of SS with BW compared to mono-digestion of SS was studied. Another objective of this study was to compare effect of source separation to mechanical treatment on AD of MSW.

2. ANAEROBIC DIGESTION

AD is a process that is done by bacteria and archaea in the absence of oxygen. It is possible to recover nutrient and energy from different kind of waste by this method such as MSW, SS, animal waste and industrial waste. AD results in production of biogas, and a valuable effluent which can be used as fertilizer (Table 1). It also reduces amount of greenhouse gases (GHG) emission but it is a slow process because of the low growth rate of microorganisms, it is pH sensitive so it needs high buffer, and high levels of ammonia can inhibit the process (Rittmann et al. 2001).

Table 1. *Advantages and disadvantages of anaerobic digestion (Rittmann et al. 2001)*

Advantages	Disadvantages
Production of biogas which can be used to produce electricity, heat, and as fuel for vehicles	Low growth rate of microorganisms
Decrease in GHG emission	High buffer requirement for pH control
Valuable effluent is obtained which can be used as a soil conditioner	Sensitivity of process to high levels of ammonia

AD has four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each stage is performed by a series of microorganisms (Figure 1). In the first step, hydrolysis, enzymes break down large macromolecules (carbohydrates, proteins, and lipids) into simple carbohydrates, amino acids, and fatty acids. Then in the second step, the products of first step are converted to organic acids and hydrogen. In the third step, fermenting bacteria converts the products of the second step to hydrogen and acetic acid. In the last step hydrogen and acetic acid are converted to methane and CO_2 by methanogens.

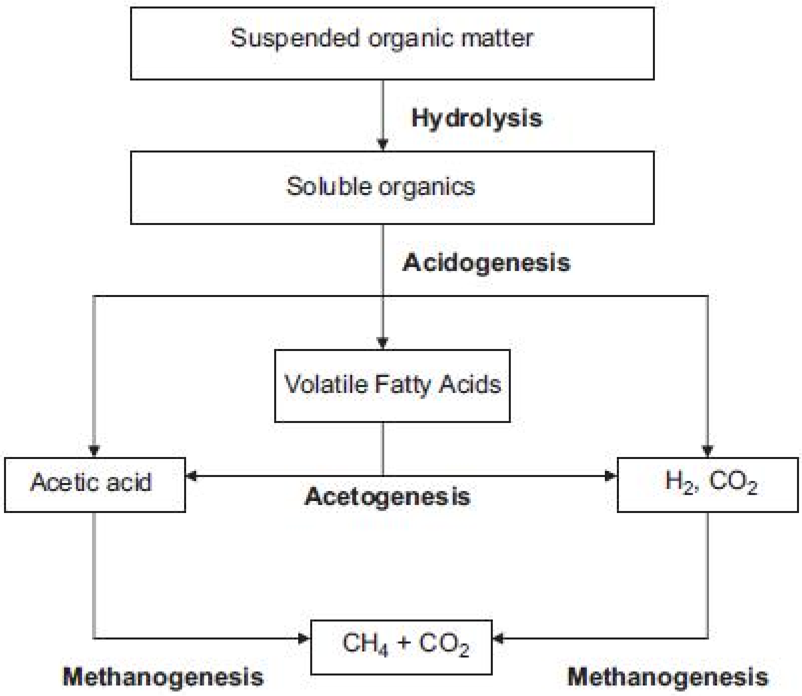


***Figure1**. Anaerobic digestion steps (Appels et al. 2008)*

2.1. Factors affecting AD

AD is a complex process which is affected by environmental factors: temperature, pH, moisture, carbon source, and nutrients.

Temperature affects AD process. Regarding temperature, AD is divided in two categories: mesophilic (30-40 °C), and thermophilic (50-60 °C). Higher temperature increases the growth rate of microorganism. Thus, thermophilic range has higher biogas production, and higher pathogen destruction while mesophilic range are more stable operation and it requires lower energy cost (Khalid et al. 2011; Rajeshwari et al. 2000)

pH is another important factor which if it is not monitored carefully can result in inhibition (usually below 7) and failure of the process (usually below 5) (Christensen 2011) . If the system is fed with too much readily degradable organics, it might result in low pH inhibition due to accumulation of VFAs. Buffer capacity of the reactor is important to neutralize any possible VFA accumulation. If the system is fed with an influent with high protein content, it is buffered against pH decrease by ammonia produced from protein, but it is susceptible to high pH inhibition caused by ammonia which will be discussed in chapter 5.2.2.

High moisture is important for AD because high water content dissolves readily degradable organic matter. Optimal moisture is in the range of 60-80 % (Khalid et al. 2011). Carbon source is necessary for growth of microorganisms. The type of carbon (organic or inorganic) and its availability can affect the AD process. Different kind of microorganisms use different type of carbon, autotrophs use inorganic carbon while heterotrophs use organic carbon. Before the AD process substrate should be characterized to determine if it has enough carbon source (Khalid et al. 2011).

Nitrogen, phosphorus, sulphur are necessary for growth of microorganism. Trace amount of heavy metals such as iron, nickel, cobalt, zinc is needed as co-factor for enzymes to have their application. Some cations are also needed which are sodium, potassium, calcium, magnesium. The optimum C:N:P:S ratio is 600:15:5:3 (Khalid et al. 2011, Rajeshwari et al. 2000).

2.2. Inhibition

Many components can have inhibitory effect on AD. Inhibition is an important factor in AD which should be paid attention carefully, because the specific growth rate of microorganisms is low and if the inhibition happens, the recovery time might be so long. Table 2 gives general methods that can be used to control inhibition in AD.

Table 2. *Possible methods to control inhibition in AD (Rittmann et al. 2001)*

Remove toxic material from waste stream
Dilute waste so toxicant is below toxic threshold
Form insoluble complex or precipitate with toxicant
Change form of toxicant through pH control

Removal or dilution of inhibitory material can be used in any situation, but dilution increases the costs because larger reactor is needed. Another way is to precipitate the toxicant for example heavy metals inhibition can be removed by addition of sulfide which form an insoluble precipitate with these metals. pH can also change the behavior of inhibitory material, for instance the equilibrium between ammonia and ammonium which will be discussed in detail later. The inhibitory materials that are discussed in this chapter are: salt, ammonia, sulfide, heavy metals, and organic compounds. (Rittmann et al. 2001)

Salt inhibition Salt inhibition is related to the cation part of the salt, rather than the anion. Sodium, potassium, calcium and magnesium are the common cations that might cause inhibition. They are needed for growth of microorganisms but excess amount causes inhibition. (Rittmann et al. 2001) They might be present in influent or be released by breakdown of organic matter or added to process for adjustment of pH. High concentrations of salts results in dehydration of bacterial cell due to osmotic pressure (Chen et al. 2008).

Ammonia inhibition Microbial community involved in AD process is important in the performance of the reactor. Concentration of ammonia is a factor that can affect microbial community and inhibit the process. Thus its concentration needs to be controlled. (Rajeshwari et al. 2000)

Ammonia nitrogen is necessary for growth of microorganism but high concentration of that might inhibit the AD process. Ammonium ion (NH_4^+) and free ammonia (NH_3) both can cause inhibition (Yenigün et al. 2013) but inhibition of ammonia is more severe and it is more often ammonia, not the ionized ammonium form, that causes inhibition (Rittmann et al. 2001). pH is an important factor in the equilibrium between ammonia and ammonium ion and the inhibitory effect which will be discussed further in chapter 5.2.2.

Ammonia and ammonium are present in the influent and also they are produced during the process by breaking down of proteins. For unacclimated inoculum concentrations of 1700-1800 mg/l of total ammonia nitrogen (i.e. free ammonia nitrogen + ammonium nitrogen) cause reactor failure, and for acclimated inoculum this concentration increases up to 5000 mg/l. (Yenigün et al. 2013)

Sulfide inhibition Sulfide is essential nutrient for growth of microorganisms and low amount is needed for a successful process. It also helps to prevent toxicity of excessive concentration of heavy metals, but high concentrations cause toxicity. Sulfide complex with heavy metals such iron, zinc or copper is not toxic. It is the soluble form that is inhibitory. (Rittmann et al. 2001)

Sulfate is present in many wastes. Sulfate is reduced to sulfide by the sulfate reducing bacteria (SRB). SRB competes with different types of microorganisms involved in AD process for the same organic and inorganic substrates and reduces the methane production. Moreover, sulfide is toxic for various microorganism groups. (Chen et al. 2008) H_2S is one of the sulfide species that is formed during AD. It is toxic to anaerobic microorganisms, it has bad odor that poses health and aesthetic problems for workers, and it is corrosive. Moreover, it is oxidized to sulfur dioxide during combustion which creates air pollution problem. (Rittmann et al. 2001)

Heavy metals inhibition Trace amount of heavy metals is need for activity of enzymes as co-factor but high concentration is toxic. Copper, nickel, zinc, cadmium, mercury, chromium, and lead can have severe toxic effect on AD (Rittmann et al. 2001). The important issue about the heavy metals is that they are not biodegradable, so there is a high possibility of their accumulation to toxic level (Chen et al. 2008).

The best solution for preventing heavy metal inhibition is increasing the quality and efficiency of separation methods at the first place in order to prevent entering them into the process. However, if they are already entered the process, using iron sulfide can be an option which makes complexes with heavy metals. (Rittmann et al. 2001)

Organic toxicants Organic compounds also can be toxic to AD process. The inhibition concentrations are different for different organic compounds. Low concentration of organic toxicant can be a source of food for anaerobic microorganisms and lead to their biodegradation and removal from the process, but accumulation of high concentrations inhibits the AD process. Chlorophenols, halogenated aliphatics, N-substituted aromatics, long chain fatty acids (LCFA), and lignins and lignin related compounds are among the organic toxicants. Other than toxicant concentration, toxicant exposure time, acclimation, cell ages, and temperature are also important in inhibitory effect. (Chen et al. 2008; Rittmann et al. 2001)

3. MUNICIPAL SOLID WASTE

MSW is the solid waste produced by households, small business places, offices and public institutions such as schools, and hospitals (Eurostat, 2012e). In other words, MSW is whatever we use in our daily life and then put it garbage. Therefore, it is very mixed waste which composed of different categories: food waste, wood and gardening waste, paper and cardboard, rubber, leather & textile, plastics, metals, and glass.

Its composition depends on several factors such as the business activities of the area, type of the food that people eat which is usually a factor of tradition and culture, religion, education level, season and climate (Das et al. 2013). For example Table 3 shows the amount and different fractions of MSW in Finland in 2012. As it can be seen more than half of the MSW generated in 2012 is mixed waste. After that, source segregated paper and cardboard and bio-waste are the most dominant ones.

Table 3. *MSW amount and fraction in Finland in 2012 (FSWA 2013)*

Type of waste	Amount (t)	Share (%)
Mixed waste	1 394 746	50.9
Paper and cardboard waste	364 902	13.3
Biowaste	363 259	13.3
Glass waste	30 476	1.1
Metal waste	123 915	4.5
Wood waste and scrap	78 563	2.9
Plastic waste	36 127	1.3
Electrical and electronic equipment	67 871	2.5
Other and unspecified	278 236	10.2
Total	2 738 095	100

MSW usually has moderate moisture content (55.9-62.9 %), high VS/total solid (TS) ratio (79-88 %), high amount of C (39.8-52.7 g/100 g TS) and low amount of N (0.48-3.2 g/100 g TS) (Table 4). Thus for AD, MSW have high organic fraction but maybe lack of nutrients (nitrogen), so with co-digestion it is possible to compensate lack of nitrogen to have higher biogas yield.

Table 4. *Characteristics of MSW*

	C (g/100 g TS)	N (g/100 g TS)	C/N	VS/TS (%)	Moisture (%)	Type of MSW	Reference
China(Beijing)	39.8	1	39.5	n.d.	62.9	Mixed	Wang et al. 2012
Haiti	52.7	1.7	31	79	55.9	Mixed	Philippe et al. 2009
Italy	48	3.2	15	88	n.d.	Source-separated OFMSW	Cecchi et al. 1986
Switzerland	39.9	0.48	83.1	n.d.	n.d.	Source-separated OFMSW	Glauster et al. 1987

n.d.: no data

3.1. MSW Separation and processing

It is possible to improve the recovery of MSW by sorting and separation, mechanically or at source. In this study two different types of MSW was used: mechanically treated and source-separated. In this regards, source separation and mechanical treatment will be discussed in the following.

3.1.1. Source separation

Source-separation is usually is the best way to achieve high quality OFMSW (Cesaro et al. 2014) which gives better results in term of biogas production and digestate quality (Bolzonella et al. 2006). The first time separation of BW experiment in Finland was done in 1982 in Joensuu, in 1988 in Vuosaari, Helsinki, in 1990-1991 in Espoo and in 1993 in Tampere, and the result was positive (Piippo 2013). So then after that, source-separation was extended to more cities and other types of waste. In many European countries, if the waste is not source separated and the organic fraction is separated by mechanical treatment, the digestate cannot be applied on soil (Banks et al. 2011).

3.1.2. Mechanical treatment

Mechanical treatment is used to recover valuable materials from waste streams. The purpose is to maximize resource recovery, prepare material for the core biological stage (Archer et al. 2005), removal of contaminating items, or separate different types of waste or homogenize the waste in order to optimize the process. Mechanical treatment can be categorized into: size reduction, separation, and compaction. (Christensen 2011)

Size reduction The aim of size reduction is to homogenize the particle distribution and increase the surface area (Christensen 2011). Smaller size of the particles makes the temperature control, moisture distribution and mixing easier, but it also can cause environmental impacts from dust and bio-aerosols, because when the size of the particle is smaller, the risk of dust or bio-aerosol generation is higher (Archer et al. 2005).

Separation Separation is the process of separating one waste stream into two or more waste streams (Christensen 2011). Screens and air classifiers can be mentioned as widely used separation techniques.

In screens separation of particles is based on the size of the openings on a screened surface. Particles smaller than a given opening, fall through the moving screen and become the fine fraction. The material contained above the screen is considered as oversize or coarse fraction. (Christensen 2011) Sieving is also a method of screening which homogenize the particles size of the waste and separate non-biodegradable materials such as plastic bags (Cesaro et al. 2014).

One of the most common used screens is trommel screen. It tumbles the waste around until it finds an open aperture in the screen and falls. Separation efficiency of a trommel screen is controlled by the size of the screen openings, the trommel diameter, the rotational speed, and the type and number of baffles. (Christensen 2011)

Air classifiers separate according to the particle's falling velocity in air stream. Less dense materials (paper, plastics, dry and light organics) are caught in the upward current of the air, while the more dense materials (metals, stones, tiles, wet organic matter) drop to the bottom. The light fraction in the air stream is often separated from air by a cyclone. (Christensen 2011)

Compaction The aim is to increase bulk density. Most waste consists of materials with low densities and large volumes, so by compaction of waste it is possible to lower transportation cost by reducing volume, reduce space required for storage, and increase the energy density of material if it is going to be used for thermal recovery. (Christensen 2011)

The compaction devices can also separate waste into two streams: an organic wet fraction and a solid dry one. The MSW is pressed with very high pressure into an extrusion chamber. The device is called pressure extruder. Organic wet fraction acts like a fluid and comes out of the extrusion holes, can be used in AD process. Solid fraction can be used in aerobic stabilization (Cesaro et al. 2014). Diameter of the extrusion hole is typically between 13-16 mm but depending on the characteristics of the treated material, it can vary. Usually smaller hole diameter gives more clean and homogenous output. (Novarino et al.2012).

3.1.3. Effect of mechanical treatment on AD

Mechanically sorted OFMSW gives lower biogas quantity and low digestate quality (Bolzonella et al. 2006). Bolzonella et al. (2006) compared the AD of source-sorted OFMSW (SS-OFMSW) and mechanically treated mixed waste. CH_4 percentage for both was 55 %. At the same range of OLR, the biogas production for SS-OFMSW (180

m^3/t waste) was much higher than biogas production for mixed waste (60 m^3/t waste) and also specific biogas production (SBP) was higher for SS-OFMSW (0.4 m^3 CH_4/kg TVS) than for the mixed waste (0.13 m^3 CH_4/kg TVS)

Agyeman et al. (2014) studied the effect of size reduction on co-digestion of food waste (FW) and dairy manure. Their results showed that by size reduction of FW particles from 8 to 2.5 mm by grinding, methane production increased by 10-29% and specific methane yields increased by 9-34%. Energy consumption for size reduction was 1.1-2.4 % of the energy provided by biogas production, so it was cost-efficient. Bruni et al. (2010) also increased methane yield by 10% in AD of biofibers separated from digested manure by size reduction to 2 mm by blender.

Mshandete et al. (2006) used 2 mm sieve after grinding of sisal fiber waste, and the methane yield increased 23% for the fine fraction in comparison to untreated fibres. Hjorth et al. (2011) studies effect of extrusion on AD of five different agricultural biomass, methane production was increased 18-70 %, which produced 6-68% more energy after subtracting the energy used by extruder. Novarino et al. (2012) also studied AD of extruded OFMSW, and their result showed reasonable biogas production, and high methane content biogas. A summary of above-mentioned effects on methane yield from different studies is shown in Table 5.

Table 5. *Effect of different mechanical treatments on methane yield*

	Technique	Increase in methane yield (%)	Reference
Size Reduction	Grinding	10-29	Agyeman et al. (2014)
	Blender	10	Bruni et al. (2010)
Separation	Sieve	23	Mshandete et al. (2006)
Compaction	Extruder	18-70	Hjorth et al. (2011)

4. SEWAGE SLUDGE

Sewage is waste matter with water carried away in sewers. So SS is the sludge from urban WWTPs (European Commission). SS can be applied on soil as fertilizer as it is rich in nutrients, but on the other hand it might contain high amount of heavy metals, xenobiotics and pathogens.

Sludge disposal to land is regulated by the EU Sludge Directive (86/278/EC). This Directive was proposed to regulate application of SS in agriculture to prevent harmful effects on soil, vegetation, animals and humans. According to European Commission sludge that has not been treated should not be used. It suggests multiple methods for treatment of SS which normally contain aerobic and anaerobic stabilization at high temperatures (thermophlic range) or conditioning with lime (European Commission 2000).

SS consists of two basic forms primary sludge and secondary sludge (Figure 2). Primary sludge is produced by applying physical or chemical treatments to remove suspended matter. One example of physical treatment is sedimentation which gravity settles suspended matter. As an example of chemical treatments coagulation can be mentioned which coagulant is added to wastewater, it neutralizes the charge of colloidal particle, and so they can accumulate and settle. (European Commission 2001) The solid content of primary sludge is about 5-10% of which 70% is organic, and in general it accounts for 60-80% of total VS of SS (Lu 2006).

Secondary sludge is mostly microbes produced by biological treatments like lagooning, bacterial beds, and activated sludge to remove the rest of organic material in wastewater. For example in activated sludge, aeration is used to reduce organic matter. (European Commission 2001) Solid content in secondary sludge is 1-6% of which about 70% is organic, and in general it accounts for 20-40% of total VS of SS (Lu 2006).

Mixed sludge is a mixture of primary and secondary sludge. If there are high amounts of nutrients in wastewater which might lead to toxicity for instance ammonia inhibition, it undergoes tertiary treatment which removes unwanted nutrients mainly nitrogen and phosphorus. (European Commission 2001) Then after that, mixed and tertiary sludge are undergone to sludge treatment, for instance anaerobic digestion, and the effluent is digested sludge.

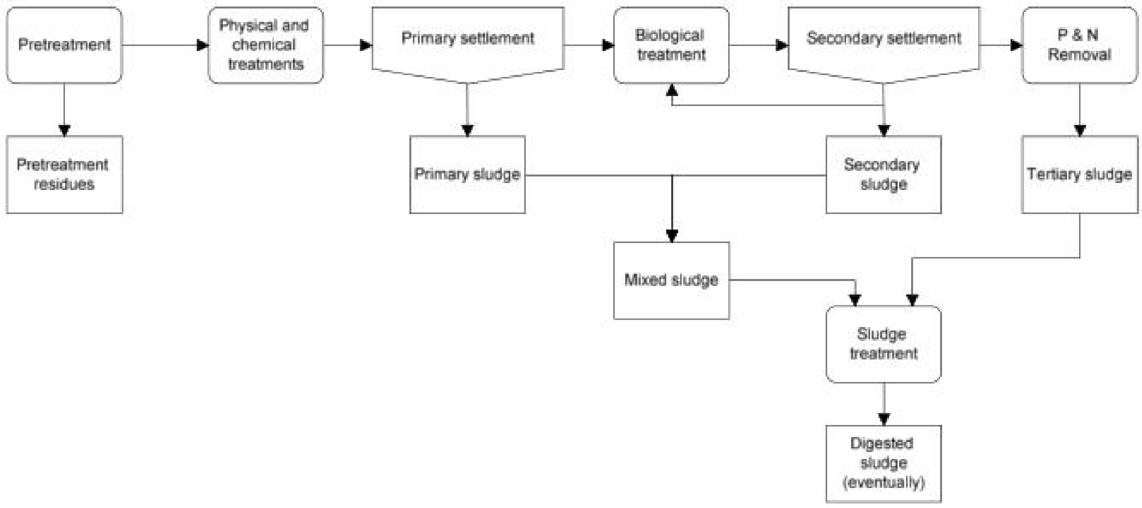


Figure 2. *Waste water treatment and sludge generation (European Commission 2001)*

SS usually has lower VS/TS than MSW (66-80 %), high moisture content (84.5 %) and it is rich in nitrogen (3.6-5.9 g/100 g TS), so it has low C/N ratios (Table 6). On the contrary, MSW has very high C/N ratios, which shows that the mixture of them can provide an optimal C/N ratio.

Table 6. *Characteristics of SS*

	C (g/100 g TS)	N (g/100 g TS)	C/N	VS/TS (%)	Moisture (%)	Type of SS	Reference
Japan	38.9	4.4	8.8	80	n.d.	Mixed sludge	Hidaka et al. 2013
Spain	24.9	3.6	6.9	73	n.d.	n.m.	Aymerich et al. 2013
China	37.2	5.9	6.3	66	84.5	Dewatered SS	Liu et al. 2012

n.d.: no data
n.m.: not mentioned

5. CO-DIGESTION

In co-digestion two or more waste streams undergo AD simultaneously. This method can improve the yields of the AD of wastes. It is a well-established process in Europe, with Germany and Scandinavia being the pioneers (Lacovidou et al. 2012). With co-digestion, it is possible to increase methane gas production, reduce the inhibitory effect of toxic compounds by diluting it, utilize the digester volume better, and save money for buying equipment for two different AD by combing them in one process. However, co-digestion might change the behavior of the material that are involved in process, or change the quality of digestate. (Montañés et al. 2013)

SS has been as co-substrate in AD with several different substrates. Dai et al. (2013) studied co-digestion of SS and FW, and their result showed improvement in stability of process, biogas production and VS destruction in comparison to mono-digestion of SS and FW. Silvestre et al. (2014) studied co-digestion of SS with grease waste, their result showed increase in OLR and methane yield in comparison to mono-digestion of SS, and the proper mixing ratio before inhibitory effect was investigated. Montusiewicz et al. (2011) studied the co-digestion of SS and intermediate landfill leachate, and their results also showed higher biogas yield in comparison to mono-digestion of SS, and co-digestion had a minor effect on increasing methane content of biogas.

MSW also has been as co-substrate for several different substrates. Shanmugam et al. (2009) studied co-digestion of MSW and leather fleshing waste, optimum C/N ratio and pH were determined, and they concluded that co-digestion of leather fleshing waste with MSW can increase biogas yield. Macias-Corral et al. (2008) studied the co-digestion of MSW with cow manure. Their result showed higher methane yield and lower weight and volume of digested residual in comparison to mono-digestion of MSW and cow manure.

Several studies (Pahl et al. 2008; Sosnowski et al. 2003; Valencia et al. 2009; Zupancic et al. 2008, etc.) have investigated co-digestion of SS and MSW. In this chapter, advantages that co-digestion of MSW and SS provides in comparison to their mono-digestion are studied. Moreover, the challenges that might occur during the co-digestion of MSW and SS are investigated and some solutions are proposed. Finally, the digestate quality is discussed and the characteristics that it should have in order to be applicable on soil are explained.

5.1. Advantages

The main advantages that co-digestion of MSW and SS provides are improvement of C/N ratio, faster hydrolysis, higher biogas production, and higher OLR and VS destruction.

C/N ratio is an important factor during AD. High C/N ratio (usually more than 30) results in low nutrient availability, microorganism will not have enough nutrients for activity and thus the methane production decreases. Low C/N ratio (usually lower than 6) means that there is not enough carbon available for microorganisms, and also it means that there might be too much nitrogen in the process which results in ammonia inhibition (Iacovidouet al. 2012). In general SS is characterized by low C/N ratio, and MSW is characterized by high C/N ratio, so it shows that mixing of them can improve C/N ratio to achieve an optimum ratio.

The rate-limiting step in the AD of SS is hydrolysis, because SS is rich in protein which their hydrolysis is time consuming. When OFMSW is added to SS, more easily degradable material are available which makes the hydrolysis faster. (Iacovidouet al. 2012)

One of the most important advantages of co-digestion is higher biogas production, higher OLR and more VS destruction, and therefore higher efficiency of the process which is illustrated in the following case study examples. Zupancic et al. (2008) studied the co-digestion of SS and OFMSW. The usual influent of digester was mixed SS (60% primary sludge, 40 % secondary sludge). The reactors were fed semi-continuously (every 3 h). In the experiment OFMSW was added to the digester influent to study the effect of co-digestion. OFMSW was domestic refuse which was collected from households. Share of OFMSW was varying between 8-28% VS during the experiment. The produced biogas was used to produce electricity and heat.

VSS degradation efficiency increased from 71% to 81%. OLR was increased by 25 %. SBP before the experiment was 0.39 m^3 kg^{-1} $VSS_{inserted}$ and at the end of the experiment was over 0.60 m^3 kg^{-1} $VSS_{inserted}$ which corresponds to 54% increase in SBP. Biogas production rate (BPR) before the experiment was 0.32 m^3 $m^{-3}d^{-1}$ and increased to 0.67 m^3 $m^{-3}d^{-1}$ which corresponds to 109% increase in BPR. Biogas quantity increased by 80 %. Electrical energy production increased by 130 % and heat production increased by 55 %.

Valencia et al. (2009) studied co-digestion of MSW and septic tank sludge of Delft, Netherlands in bioreactor landfill simulators. MSW was shredded before feeding. Share of MSW was 75% wet weight. Biogas production in both reactors (mono-digestion of MSW and co-digestion of MSW with septic tank sludge) was similar during the first 100 days. But then the reactor with a mixture of MSW and sludge produced more biogas. Biogas yield for mono-digestion of MSW was 0.32 m^3 biogas/kg of $VS_{inserted}$

while biogas yield for co-digestion was 0.60 m^3 biogas/kg of $VS_{inserted}$ which corresponds to 87% increase in biogas yield of MSW as a result of co-digestion with septic tank sludge. The reactor with MSW+S has had 60% higher VSS destruction, thus more stabilized waste.

Moreover, these benefits can be seen in Sosnowski et al. (2003) study. The experiment was done in semi-continuous reactors (once per day feeding). SS was mixture of primary sludge and thickened excess activated sludge 1:1 volume. OFMSW was source-separated kitchen waste which was grounded before feeding. Mixing ratio of co-digestion was 75% volume SS, and 25% volume OFMSW. SBP in the reactor containing mono-digestion of OFMSW was 0.419 dm^3/g VSS_{added} and in the co-digestion of OFMSW and SS reactor was 0.532 dm^3/g VSS_{added} which shows 27% higher SBP in co-digestion of OFMSW and SS in comparison to mono-digestion of OFMSW.A summary of the above-mentioned benefits can be seen in Table 7.

Table 7. *Effect of co-digestion of OFMSW with SS on AD*

Reference	Δ (OLR)	Δ (Biogas quantity)	Δ (BPR)	Δ (SBP)	Δ(VSS destruction)	Share of MSW
Zupancic et al. (2008)	+ 25	+ 80	+ 109	+ 54	+ 10	8-28% VS
Valencia et al. (2009)	n.d.	+ 68	n.d.	+ 87	+ 60	75% wet weight
Sosnowski et al. (2003)	n.d.	n.d.	n.d.	+ 27	n.d.	25% vol.

Δx = change of x as a result of co-digestionof MSW and SS as percentagein comparison to mono-digestion

n.d. = no data

BPR=biogas volume/ reactor volume/ time

SBP= biogas volume / inserted VSS mass

5.2. Challenges and solutions

The most important challenges that are faced in co-digestion are: mixing ratio, ammonia inhibition, acidification, salt toxicity, impurities, and heavy metals inhibition.

5.2.1. Mixing ratio

Mixing ratio is an important factor which needs to be considered carefully, as the wrong mixing ratio can result in lowering the efficiency of process or failure of that. The optimum mixing ratio differs from one system to another (Iacovidouet al. 2012). For example, Mata-Alvarez et al. (2000) stated the optimum mixing ratio for SS (65% raw primary sludge, 35% thickened activated sludge) and OFMSW as 75:25 , Demirekleret al. (1998) recommended a ratio for primary sludge: OFMSW of 80:20, Kim et al. (2007) stated the optimal mixing ratio for SS and FW as VS ratio of 60:40.

If too much SS in influent is used, most probably ammonia toxicity and lack of enough carbon sources will limit the process. And if too much OFMSW in influent is used then there is a risk of acidification or salt toxicity, due to high amount of readily available organic matter and salts.

5.2.2. Ammonia inhibition

SS has high nitrogen content, so if too much SS is used in co-digestion with MSW, there is a risk of ammonia inhibition. Ammonia and ammonium ion are both toxic and their existence depends on the pH and temperature. Ammonia is much more toxic because it can pass through cell membranes and cause proton imbalance and potassium deficiency. (Appels et al. 2008) The following reaction shows the equilibrium between ammonia and ammonium ion:

$NH_4^+ \leftrightarrow H^+ + NH_3 \quad K_a = 5.56 * 10^{-10}$ (Rittmann et al. 2001)
$pH = 9.26 + \log [NH_3]/[NH_4^+]$

At pH higher than 8, the equilibrium shifts toward ammonia generation. Thus for controlling inhibition, one option could be to keep pH lower than 8, then equilibrium is toward ammonium ion generation which its inhibition effect is minor. (Rittmann et al. 2001)

At higher temperature, the dominant one is free ammonia so thermophilic process is more susceptible to ammonia inhibition (Appels et al. 2008), and decreasing the temperature can overcome ammonia inhibition (Yenigün et al. 2013). Another option to prevent toxicity is adaptation of microbial community gradually to higher concentration of ammonia. Sung et al. (2003) reported no inhibition up to 2000mg-N/l under thermophilic condition as a result of acclimation of methanogens.

Pre-treatment of substrate by struvite precipitation or air stripping or membrane can be other options to prevent ammonia toxicity. Zhang et al. (2012) studied the effect of ammonia removal by air stripping in AD of piggery wastewater. Methane production increased from 0.23 L CH_4/ L d of the control to 0.75 L CH_4/ L d at pH 9.5, and the authors suggested air stripping as a viable option for overcoming ammonia inhibition. Lauterbock et al. (2012) studied effect of membrane contactors for ammonia removal in AD of slaughterhouse wastes. A hollow fiber membrane contactor was used. Concentration of free ammonia was reduced by 70% and higher biogas yield was observed.

Dilution of substrate is another recovery method for ammonia toxicity, but it increases reactor size and consequently the cost of the process (Tada et al. 2005). Nielsen et al. (2008) diluted the biomass from inhibited cattle manure digestion process with NH_4Cl with water, or digested manure, or fresh manure. The process was recovered after 31 days and the highest methane production rate was for dilution with fresh cattle manure.

Moreover, changing C/N ratio by changing mixing ratio and addition of compounds like zeolite, and activated carbon are other options to prevent or overcome ammonia inhibition (Rajagopal et al 2013). Zeshan et al. (2012) stated C/N ratio of 32 resulted in 30% less ammonia formation in digester in comparison to C/N ratio of 27, and recommended adjustment of C/N ratio as a method to reduce or overcome ammonia inhibition. Tada et al. (2005) studied the effect of zeolite on ammonium removal in AD of SS and they stated that 5% (w/w organic waste) and 10% natural mordenite can result in enhancing AD process and biogas production. Hansen et al. (1999) increased the methane yield in AD of swine manure from 67 ml CH_4/g-VS to 126 ml CH_4/g-VS by addition of 1.5% (w/w) activated carbon.

5.2.3. Acidification

As it was previously mentioned addition of easily degradable matter in OFMSW provides faster hydrolysis. As a result of faster hydrolysis, more organic acids are produced in shorter time. These acids are used by microorganisms, but if the rate of their production is higher than the rate of their consumption by microorganism, acidification occurs. Acidification results in inhibition or even completely failure of the process.

Wang et al. (2009) studied the effect of VFAs on methane yield. Acetic acid, propionic acid, butyric acid and ethanol were used as substrates. In this study the concentration of ethanol, acetic acid and butyric acid could be increased up to 2400, 2400, and 1800 mg/l without significant inhibition on methanogenesis but propionic acid at concentration of 900 mg/l caused significant inhibition. The optimum concentration of ethanol, acetic acid, propionic acid and butyric acid for highest methane yield was proposed as 1600, 1600, 300 and 1800 mg/l. Fast hydrolysis might also result in LCFA accumulation which will also have the inhibition effect (Iacovidouet al. 2012).

Angelidaki et al. (1992) reported inhibition of AD of cattle manure at low concentration LCFAs oleate and stearate (0.5 g/l for oleate and 1.0 g/l for stearate). LCFAs are adsorbed to cell membrane or cell wall of microorganisms and interfere with transportation or protection of cell. LCFA inhibition on sludge depends more on physical characteristics of the sludge rather than biological characteristic. Acidification can be controlled by constant pH control, and adjusting the pH by alkaline solutions if is necessary. Calcium addition may also reduce LCFA inhibition (Hanaki et al. 1981).

5.2.4. Salt Toxicity

Generally MSW consists of high amount of FW, around 40-50 %. High salt content (mainly NaCl) is a characteristic of FW. (Dai et al. 2013) So MSW has usually high salt contents, and salt toxicity can inhibit the AD process during its co-digestion with SS (Suwannoppadol et al. 2012). Moreover, due to pH changes during the process, cation part of the salt might be added to system in order to modify the pH and avoid acidification. High concentrations of salts decrease cell activity and results in dehydration of bacterial cell because of increase in osmotic pressure. (Chen et al. 2008; Suwannoppadol et al. 2012) and inhibits the AD process.

One of the ions that is of high concern in AD of MSW is sodium (Suwannoppadol et al. 2012). If a cation for example sodium is in an inhibitory concentration, addition of another cation such as potassium may relieve the inhibition (Rittmann et al. 2001) but in Vyrides et al. (2010) study, addition of potassium had slight decrease in sodium inhibition. Another way is acclimation of substrate to high concentration of salts, but it might take long time. Mendez et al. (1995) reported 9 months as required time for acclimation for seafood-processing wastewater.

Suwannoppadol et al. (2012) stated using grass leaves as an approach to overcome sodium toxicity, and it was concluded that it is a cost-effective method. Vyrides et al. (2010) reported minor effect of trehalose, and N-acetyl-β-lysine on decreasing sodium inhibition, but strong effect of glycinebetaine on overcoming sodium inhibition. However using glycinebetaine for decreasing sodium inhibition in commercial scale is considered costly (Suwannoppadol et al. 2012).

5.2.5. Impurities

Impurities found in OFMSW such as plastics, metals, and glass might cause technical malfunctioning in co-digestion facilities. Plastics in the form of plastic bags can be wrapped around the stirring equipment in storage and reactor tanks, and cover the pumps. Furthermore, some plastics like phthalates can change the quality of the digestate and it cannot then be applied on soil as fertilizer. (Hartmann et al. 2004) Impurities in form of metals can result in clogging (Iacovidouet al. 2012). In order to prevent such impacts the quality and efficiency of separation needs to be increased.

Weiland (2000) reviewed that BW from MSW may contain high amount of impurities such as plastics, metals, and sand, and considered pre-treatment necessary for AD of MSW or its co-digestion. It is proposed that plastics and sand can be separated by floatation and sedimentation, and size reduction is necessary for AD of MSW. Zhang et al. (2007) removed impurities such as wood, metal, cardboard, glass and plastics from food waste by screening and grinding.

5.2.6. Heavy metals inhibition

SS can contain high concentration of heavy metals (Chen et al. 2008), so heavy metals inhibition in co-digestion of SS with MSW can occur. Using iron sulfide can be an approach to relieve the inhibition. Iron is generally nontoxic and sulfide make complex with heavy metals which is not toxic. (Rittmann et al. 2001)

Pahl et al. (2008) studied the anaerobic co-digestion of mechanically biologically treated MSW (dry screening process employing a bag splitter, 60 mm trommel, over-band magnet and Eddy current, composting in a bunker system for about 2 weeks, maturation for 6 month and final screening) with primary SS. The methane content of the biogas was low in all cases: 40–50% methane in biogas compared to 55–70% reported in other studies (Davidsson et al. 2007; Hartmann and Ahring, 2005).

Concentrations of heavy metals (cadmium, copper, chromium, nickel, lead, zinc and mercury) were determined according to the guidance on methods of sampling and analysis UK Environment Agency's National Laboratory Service ('Blue Books'; EA, 2007). Concentration of Zn, Cr, Cu were higher than EC50 values (the concentration that causes 50% inhibition of the process). It can be suggested that because methanogens are the most sensitive microorganisms in the process, heavy metals has affected them the most so they have produced less methane gas.

5.3. Use of digestate

The digestate should pass EU end-of-waste (EoW) criteria. EoW criteria determine when a waste stops to be waste and gets value of a product (European Commission 2008). The aim is regulation of recycling and recovery activities in EU.

According to Article 6 (1) and (2) of the Waste Framework Directive 2008/98/EC, a waste stops to be waste when it undergoes a recovery method and meets the following conditions:

- There should be a special application for the substance.
- There should be a demand for that substance.
- The use of substance should not be illegal and meets the existing regulations and legislations.
- The use of the substance should not have negative environmental and health effect.

The digestate of anaerobic co-digestion of MSW and SS is rich in nitrogen and phosphorous, and can be applied on soil as fertilizer but it needs to meet these criteria in order to be applicable. These criteria have been conducted by the Joint Research Centre. The study on biodegradable waste subjected to biological treatment (compost/digestate) and waste plastics is in progress. A Draft Final Report for biodegradable waste has been proposed on July 31, 2013.

Digestate of anaerobic co-digestion of MSW and SS meets the first two requirements, because it is commonly used for a specific purpose and the purpose is fertilizing the soil, agriculture is the existing market for that. But before its application on soil it needs to meet the last two requirements. Summary of some of the legislations and limit values for use of digestate as fertilizer are shown Table 8. European countries have their own national regulations on use of digestate as fertilizer (Table 8). EU end of waste collects the regulations from different countries and analyze them, to set uniform limits.

Table 8. *Digestate quality for applying on soil*

	England	Sweden	Germany	Belgium	EU EoW
Heavy metals (mg/ kg dry matter)					
Lead	200	100	150	300	120
Cadmium	1.5	1	1.5	6	1.5
Copper	200	600	100	375	200
Chromium	100	100	100	250	100
Mercury	1	1	1	5	1
Nickel	50	50	50	50	50
Zinc	400	800	400	900	600
Impurities (% of the dry substances weight)					
plastics, glass, metals, and composites	0.5	0.5	0.5	0.5	0.5
Pathogen indicators					
E.coli (CFU/g fresh matter)	1000	1000	n.d.	n.d.	1000
Salmonella	Absent in 25 g fresh matter	Absent in 25 g fresh matter	Not traceable	Absent	Absent in 25 g fresh matter
Organic substances and pollutants					
Organic substances[a] (%)	n.d.	20	30	n.d.	15
Organic pollutants[b] (mg/kg dry matter)	n.d.	n.d.	n.d.	15.78	6
Reference	UK PAS 110	Swedish SPCR 120 QAS	German RAL GZ 245	Belgian VLACO QAS	EU End of Waste Criteria

a) Measured as loss on ignition in percent of the dry substance weight

b) Polycyclic aromatic hydrocarbons: naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene,pyrene, benzo[a]anthracene,chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrenedibenzo[a,h]anthracene, benzo[ghi]perylene

n.d. = no data available

6. MATERIALS AND METHODS

6.1. Substrates and inoculum

OFMSW was sampled from Stormossen, Vaasa, Finland. It was shredded and sieved in the plant. Source-separated BW was sampled from Tampere waste treatment plant (Tarastenjärvi). Then they both (OFMSW and BW) were homogenized using a kitchen meat grinder in laboratory, and kept at 8 °C before using.

SS was sampled from Tampere WWTP (Viinikanlahti). SS was a mixture of primary and secondary sludge. After sampling from WWTP and transportation to the laboratory, it was kept at 8 °C before using. Inoculum was digestate sludge from biogas plant in Tampere WWTP. Characteristics of the substrate are shown in Table 9.

Table 9. *Substrate characteristics*

	OFMSW			BW			SS		
	DM(g/kg DM)	FM (kg/tn)	FV (kg/m^3)	DM(g/kg DM)	FM (kg/tn)	FV (kg/m^3)	DM(g/kg DM)	FM (kg/tn)	FV (kg/m^3)
Water Soluble Nitrogen	3.98	1.21	1.10	1.19	0.389	0.396	8.83	0.281	0.284
Total Nitrogen	26	8.05	7.25	22	7.15	7.25	47.5	1.5	1.55
Soluble Phosphorus	1950	585	530	1700	545	555	81	2.6	2.6
Total Phosphorus	3,5	1.1	1	3.3	1.05	1.1	18.3	0.55	0.6
Volume weight	n.a.	n.a.	900	n.a.	n.a.	1000	n.a.	n.a.	1000
Dry matter	n.a.	30.55%	n.a.	n.a.	32.55%	n.a.	n.a.	3.15%	n.a.

DM: in dry matter
FM: in fresh matter
FV: in fresh volume
n.a.: not applicable

6.2. Batch experiments

Biochemical methane potential (BMP) assays were carried out in 1 L glass bottles with a working volume of 700 ml. Samples were inoculum (350 g), OFMSW (14.9 g), BW (15.7 g), and SS (183.2 g), duplicate each sample. Inoculum to substrate VS ratio was 1. Tap water was added to get the final working volume. $NaHCO_3$ (4 g/l) was added as a buffer. Then bottles were flushed with N_2 for 3 min to make anaerobic condition and were sealed with silicon stoppers.

Bottles containing only inoculum and water were used as control. Amount of methane produced in assays with inoculum was subtracted from methane produced in assays with inoculum and substrates. The prepared assays were incubated at 35 ° C for 28 days. Biogas produced was collected in 5 L aluminum gas bags. The content of each bottle was mixed by hand before each gas sampling. The gas samples were taken from a rubber septum 2 times per week for the first two weeks and once per week for the last two weeks.

6.3. Reactor experiments

Four semi-continuously fed CSTRs were used in this experiment. Each reactor had a total volume of 6 L and liquid volume of 5 L at 35 °C. Five liters of inoculum was added to each reactor on the first day. The contents of each reactor are shown in Table 10.

Table 10. *Contents of the four studied reactors (VS %)*

Reactor/substrate	OFMSW	BW	SS
R1	100	-	-
R2	-	50 (day1-42) 71.5 (day 43-90)	50 (day 1-42) 28.5 (day 43-90)
R3	-	100	
R4	-	-	100

BW to SS VS ratio in R2 was 1:1 for the first 42 days and then changed to 2.5:1 for the rest of the experiment (day 43-90). The OFMSW and BW for reactors 1 and 3 were diluted with tap water to achieve the desired hydraulic retention time (HRT). OLR in R1, R2, and R3 was 1 kg VS/m^3 d for 65 days which then increased to 2 kg VS/m^3 d (day 65-90). In R4 due to reactor leaking, the data until day 47 was excluded from results. The OLR was 0.3 kg VS/m^3d for 4 days (day 47-50) and after that it was increased to 1.1 kg VS/m^3d until the last day.

Temperature in the reactors was maintained by a water circulation heater. Reactor content was mixed by a mixer (26 rpm) with a timer (30 min on/30 min off). Feeding was done manually five days per week. Before each feeding the same amount of digestate was taken out. The produced biogas was collected in 10 L aluminum gasbags.

6.4. Analytical methods

Gas composition (CH_4 , CO_2 , N_2) was measured by a gas chromatograph (Schimadzu GC-2014, Agilent Porapak column 1.8 m*2.00 mm) with a thermal conductivity detector (TCD). Helium was used as carrier gas, the operating conditions were: oven 40 °C, detector and injection port 80 °C. The biogas volume was measured by water displacement method. Biogas volumes were converted to standard temperature and pressure conditions (STP, T= 273.15 K, p= 1 bar)

pH was measured with a WTW pH3210 pH-meter immediately after taking out the digestate. Soluble chemical oxygen demand (SCOD) was measured according to Finnish Standard Methods (SFS 5504, Finnish Standard Association, 1988). The samples for SCOD analysis were filtered through glass microfiber filters (diameter 47mm). TS and VS were analyzed according to standard methods (APHA, 1998).

VFAs were determined by using a gas chromatograph (Schimadzu GC-2010, ZB-WAX column 28.6 m*0.25 mm). Helium was used as carrier gas and operating conditions were: oven 50 °C, detector and injection port 90 °C. The samples for VFA analysis were filtered through Porvair syringe filters (30 mm diameter, 0.22 µm pore size). In conversion of VFA concentrations to SCOD, the following coefficients were used: acetic acid 1.066, propionic acid 1.512, isobutyric and butyric acid 1.816, valeric acid 2.036, and caproic acid 2.204. Total ammonia nitrogen (TAN) was determined according to standard method (substrates, method 984.13, AOAC, 1990).

7. RESULTS

7.1. Reactor experiments

7.1.1. OLRs and methane production

Co-digestion of BW and SS and mono-digestion of OFMSW, BW, and SS were studied in semi-continuous CSTR reactors for 90 days at 35 °C. The results for methane yield, OLR, and methane content are presented in Figure 3, and TS and VS of the substrates and effluents are shown in Table 11.

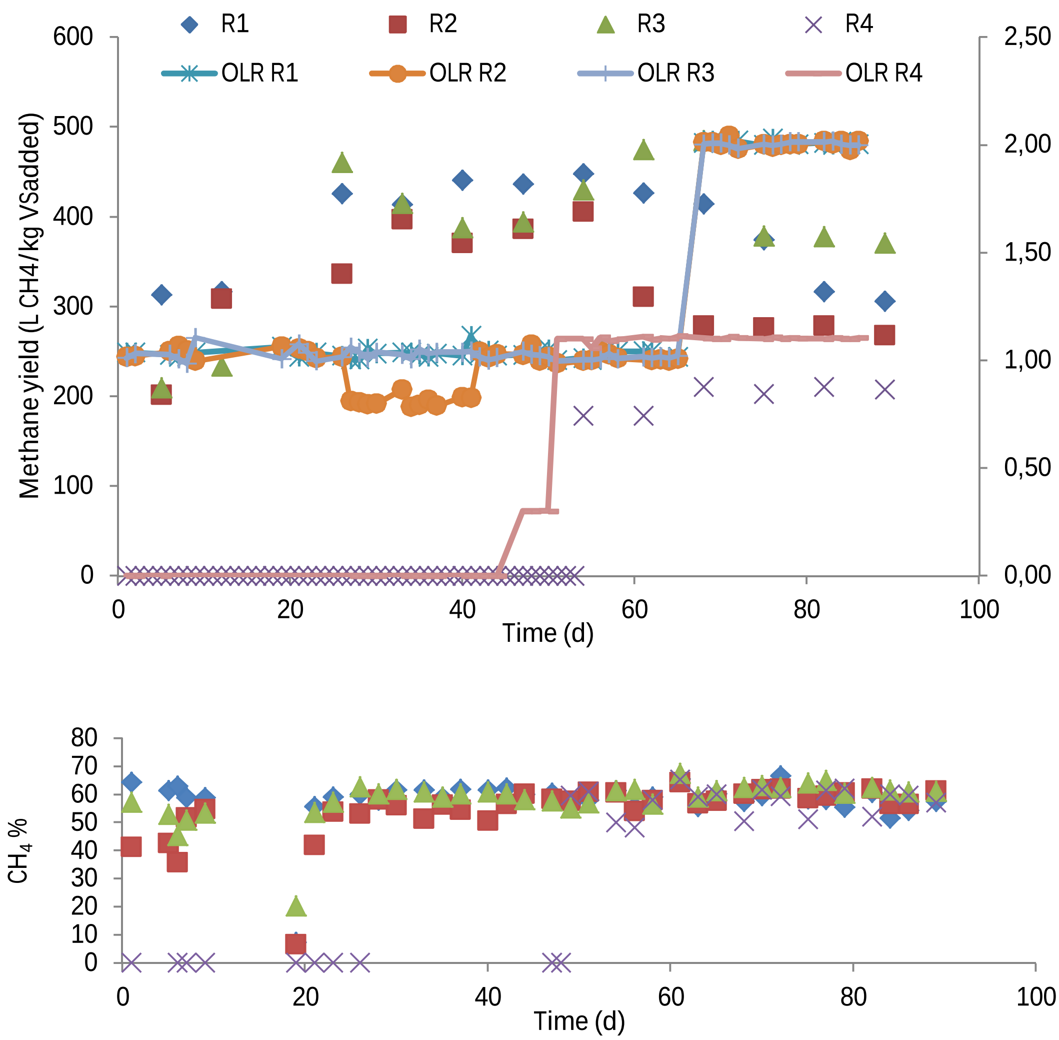


Figure 3. *Methane yields, OLRs, and methane content of the four studied reactors (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

OLR for OFMSW (R1), BW+SS (R2) and BW (R3) was 1 kg VS/m^{3}d for 65 days and then increased to 2 kg VS/m^{3}d until the last day (day 90). In R2 days 27-41 OLR was 0.8 due to decrease in amount of SS in order to increase HRT to 20 days which then again increased to 1 until day 65 by increasing the amount of BW. The highest weekly average methane yield for OFMSW was 447 L CH_4/kg VS_{added} (week 8) for BW+SS was 405 L CH_4/kg VS_{added} (week 8) and for BW was 485L CH_4/kg VS_{added} (week 10) which were observed when the reactors were operated with an OLR of 1 kg VS/m^{3}d.

Methane production started immediately in all reactors except SS reactor, in which the biogas yield and methane content were low. The highest weekly average methane yield for SS was 210 L CH_4/kg VS_{added} (week 10) and it was observed when the reactor was operated with an OLR of 1.1 kg VS/m^{3}d. Methane content of the biogas in all of the reactors was 55-63 %, except day 19 which the reactors were opened to change the sealing. After that day, the methane content could increase up to 60 % in 4 days.

OFMSW and BW respectively had the highest average methane yield (386 L CH_4/kg VS_{added} and 385 L CH_4/kg VS_{added}). SS had the lowest average methane yield (198 L CH_4/kg VS_{added}). Co-digestion of BW and SS had higher methane yield (318 L CH_4/kg VS_{added}) than mono-digestion of SS.

Table 11. *TS and VS of substrates and the last day effluent (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

	Substrates				Last days effluent			
	OFMSW	BW	SS (day 1-50)	SS (day 50-90)	R1	R2	R3	R4
TS	30.9	30.2	0.6	3.8	3.9	4.4	3.5	4.1
VS	26.3	24.8	0.6	2.2	2.9	2.4	2.4	1.4
VS/TS	85.2	82.1	65.3	59	74.1	53.6	67.3	34.4
VS removal					66	85	71	36

OFMSW had the highest VS/TS ratio (85.2%). VS/TS ratio of BW (82.1%) was close to OFMSW. SS had the lowest VS/TS ratio (65.3% first batch, and 59% second batch). R2 (BW+SS) had the highest VS removal (85%). R1 (OFMSW) and R3 (BW) had VS removal of 66% and 71% respectively. R4 (SS) had the lowest VS removal (36%).

7.1.2. VFA and SCOD

For SS (R4), the VFAs are reported after day 49 (Figure 4). The highest TVFA for OFMSW (R1) was 75 mg/l (day 42) and for SS (R4) was 25 mg/l (day 90). In BW+SS reactor (R2) there was a sudden increase to 1157 mg/l (day 49) which was due to accumulation of acetic acid and butyric acid. For BW (R3) TVFA increased from 219 mg/l to 909 mg/l and it decreased gradually to 46 mg/l at the end of the experiment.

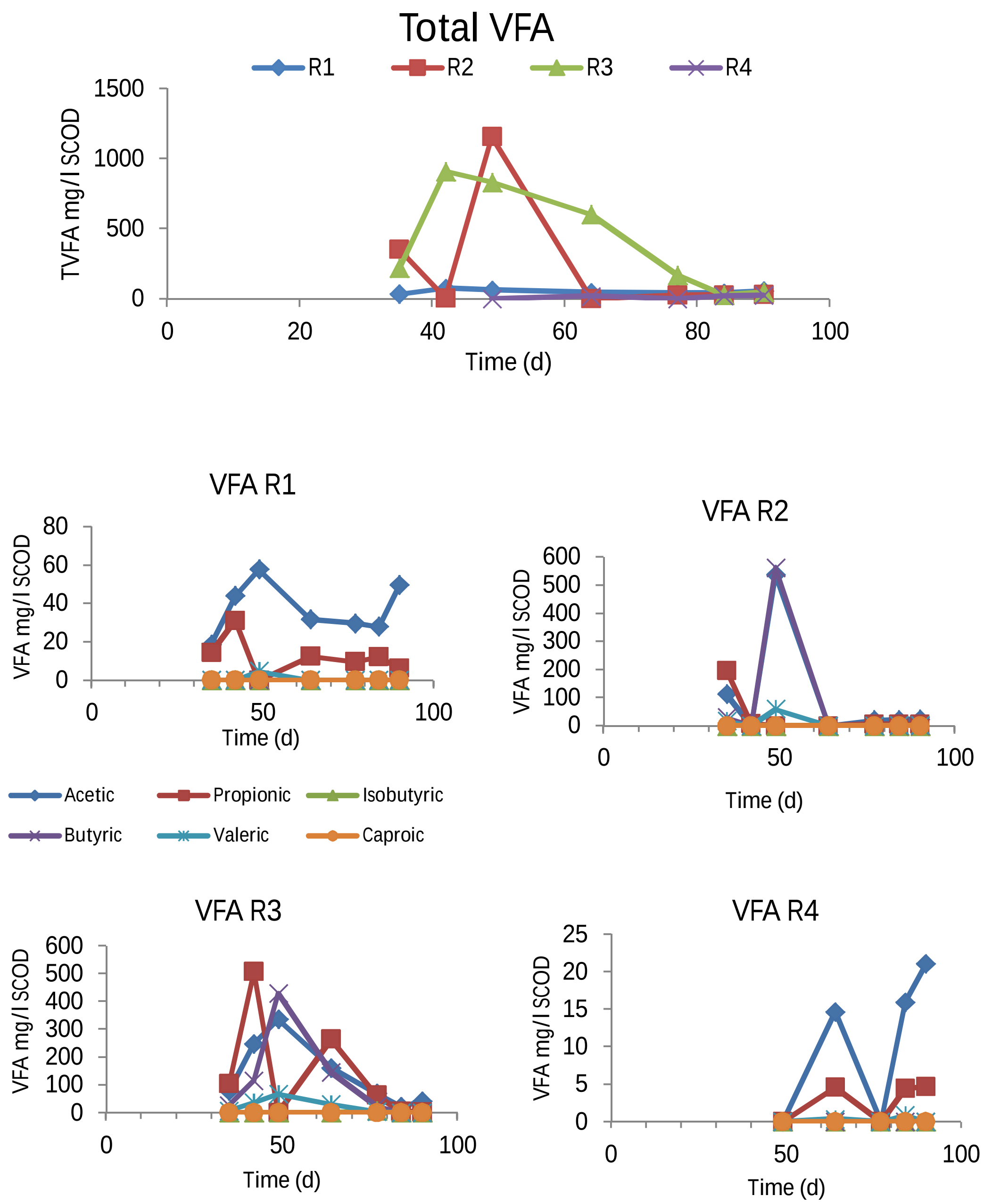


***Figure 4.** VFA accumulation in four studied reactors (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

In the day 49 there was accumulation of acetic acid in OFMSW, BW+SS, and BW reactors (Figure 4). By increasing the OLR to 2 kg VS/m^{3}d in OFMSW, BW+SS, and BW reactors there was not any significant VFAs accumulation. In SS reactor, after increasing the OLR to 1.1 kg VS/m^{3}d an increase in concentration of acetic acid and propionic acid was observed (day 64).

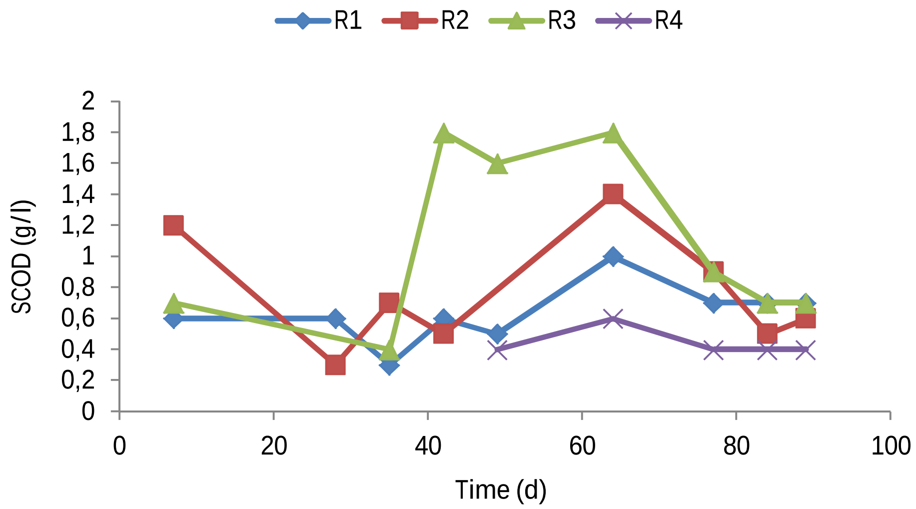


Figure 5. *SCOD in four studied reactors (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

For SS reactor, the SCODs are reported after day 49. The highest SCOD for OFMSW was 1 g/l, for BW+SS was 1.4 g/l, for BW was 1.8 g/l, and for SS was 0.7 g/l (Figure 5). Concentrations of SCOD were changed more in R2 and R3. After day 35, that process was more stable, BW had higher concentrations of SCOD in comparison to other reactors. SS had the lowest concentration of SCOD.

7.1.3. Ammonia and pH

TAN concentrations in all of the reactors were lower than 1 g/l (Table 12). OFMSW had the highest TAN concentration (843 mg/l), SS had the lowest TAN concentration (581 mg/l), and concentrations of TAN in BW, and BW+SS reactors were equal (829 mg/l) and between TAN of OFMSW and SS.

Table 12. *TAN of the last day effluent of the four studies reactors (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

	R1	R2	R3	R4
TAN (mg/l)	843	829	829	581

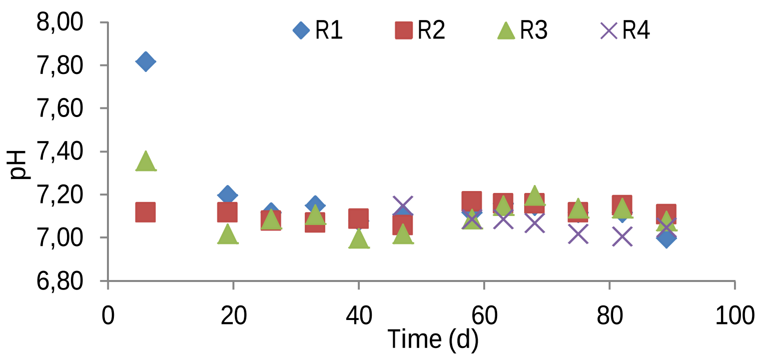
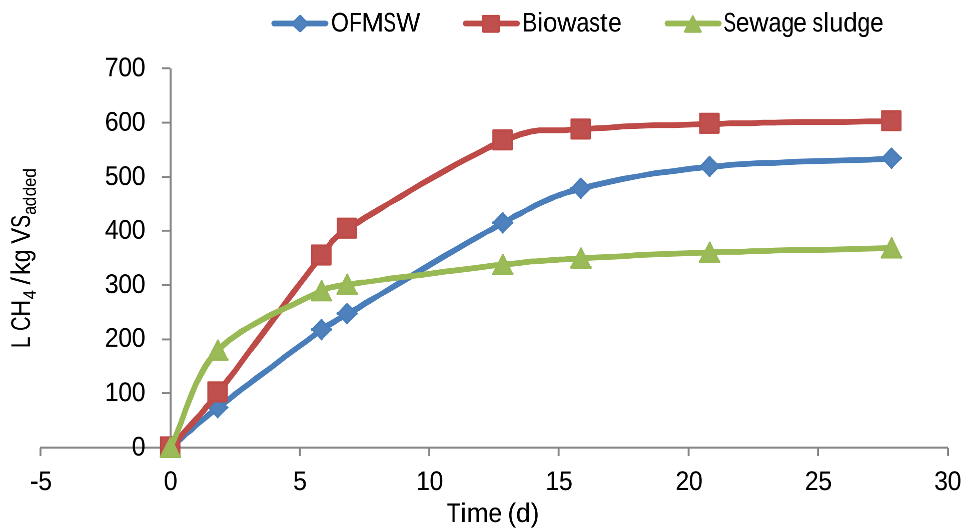


Figure 6. *pH in four studied reactors (R1: OFMSW, R2: BW+SS, R3: BW, R4: SS)*

pH in all of the reactors was not fluctuating too much, and it was in a normal range between 7.0 -7.2 (Figure 6) except OFMSW day 6 which was 7.82 and BW day 6 was 7.36 which then decreased to 7.20 and 7.02 respectively.

7.2. Batch experiment

In this experiment methane yields for the substrates and cumulative methane productions for substrates and inoculums were determined (Figure 7). BW had the highest methane yield of 603 L CH_4/kg VS_{added}. Methane yields for OFMSW and SS were 534 and 369 L CH_4/kg VS_{added} respectively. BW with producing 2.65 L CH_4 had the highest cumulative methane production. Cumulative methane productions of OFMSW and SS were 2.36 L CH_4 and 1.71 L CH_4 respectively.



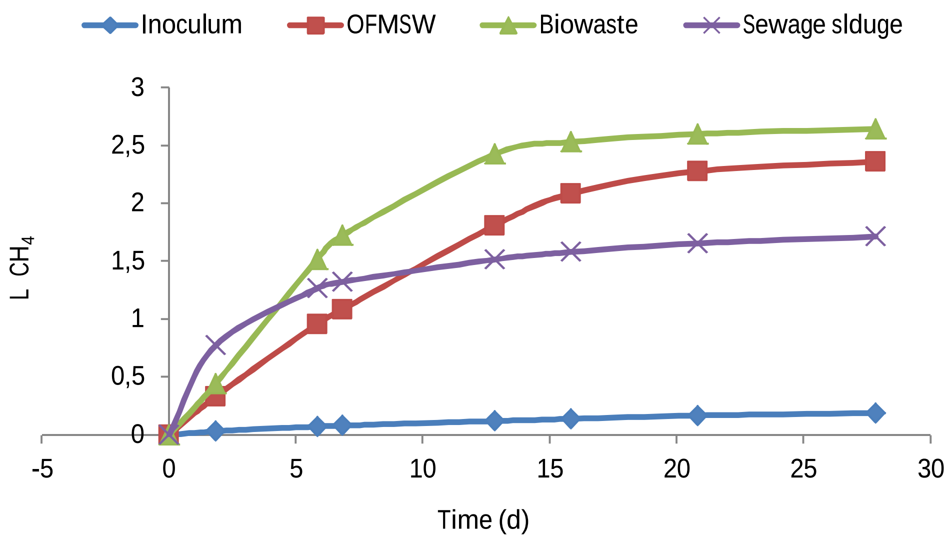


Figure 7. *Methane yield of substrates, inoculum reduced (upper) and cumulative methane production of substrates and inoculums, inoculum not reduced (lower)*

8. DISCUSSION

An increase in OLR from 1 to 2 for OFMSW, BW+SS, and BW reactors resulted in decrease in methane yield. The reason could be either the optimum OLR is somewhere between 1 and 2 or higher OLR results in slower biogas production (Sosnowski et al. 2003), so if the experiment would continue it might resulted in higher yields. An increase in OLR from 0.3 to 1.1 for SS reactor resulted in increasing of methane yield, which shows that there was not any overloading.

OFMSW had the highest average methane yield in this study (386 L CH_4/kg VS) and then BW was in the second order regarding the methane yield (385 L CH_4/kg VS) and its methane yield was so close to OFMSW. Co-digestion of SS and BW had the third highest average methane yield (318 L CH_4/kg VS), and SS had the lowest methane yield in this study (198 L CH_4/kg VS). Methane content of all of the reactor (55-63 %) was similar to other studies (Cavinato et al. 2013; Davidsson, et al. 2007; Kim et al. 2011)

Methane yield OFMSW and BW samples were similar to other studies. Methane yield of yard waste sample was reported as 345 L CH_4/kg VS (Lissens et al. 2004) and methane yield of source-segregated FW was reported as 402 L CH_4/kg VS (Banks et al. 2011). Methane yield of SS (198 L CH_4/kg VS) was between the reported yields of 116 and 318L CH_4/kg VS reported for similar material by Kim et al. (2003) and Sosnowski et al. (2008) respectively.

Methane yield of co-digestion of SS and BW in this study (318 L CH_4/kg VS) was similar to the yield of 340 L CH_4/kg VS for co-digestion of SS and source-sorted BW in mesophilic conditions (37 °C) and OLR 1.2 kg (Cavinato et al. 2013) and yield of 350 L CH_4/kg VS for co-digestion of BW and SS at mesophilic conditions and OLR 3 kg VS/m^3 d (Yu et al. 2014)

BW+SS reactor (R2) produced more biogas than mono-digestion of SS (R4) which is in line with other studies which reported increase in biogas produced from AD of SS as result of co-digestion with MSW (Sosnowski et al. 2003, Zupancic et al. 2008). BW+SS reactor had also 61% higher methane yield than mono-digestion of SS, similar to 54% increase in methane yield of SS reported as a result of co-digestion with MSW (Zupancic et al. 2008).

VS/TS of OFMSW and BW in this study (85.2%) was similar to VS/TS ratio of 91.8% reported for OFMSW (Cabbai et al. 2013) and VS/TS of 84.5% reported for source-separated BW (Cavinato et al. 2013). High VS/TS ratio of OFMSW and BW supports high methane production and methane yield of those two substrates. VS/TS of the SS samples (65.3 % and 59 %) were a bit lower than VS/TS of 69 % and 73% reported for SS by Bolzonella et al. (2006) and Montusiewicz et al. (2011) respectively.

VS removal of OFMSW reactor (R1) and BW reactor (R3) were close together (66% and 71% respectively) and similar to VS removal of 65% reported for organic fraction of household waste at 35 °C (Gallert et al. 1997). BW+SS reactor (R2) had high VS removal (85%) which shows the good performance of the reactor and positive effect of co-digestion on VS removal. This increase in VS removal as a result of co-digestion was higher than the 10% increase in VS removal reported for SS as a result of co-digestion with domestic organic waste (Zupancic et al. 2008).

In SS reactor (R4) VS removal was quite low (36%). Reason could be poor performance of the reactor but usually digestion of SS results in low VS removal, and it shows that VS in SS are not well biodegradable. For instance, VS removal of mixture of primary and secondary SS in Montusiewicz et al. (2011) study was 34.4 %.

Maximum TVFA concentration for SS in this study (26 mg/l SCOD) was lower than TVFA of 42 mg/l SCOD reported for SS (Bolzonella et al. 2006). Maximum TVFA for OFMSW in this study was 75 mg/l SCOD while TVFA concentration in AD of OFMSW has been reported in the range of several thousand mg/l (Pahl et al. 2008).

Although accumulation of acetic acid was observed in some days (day 49 in OFMSW, BW+SS and BW reactor, days 64 and 90 in SS reactor), in all of the reactors concentration of acetic acid was lower than 2400 mg/l which is considered as the inhibitory concentration of acetic acid for methanogens (Wang et al. 2009). Acetic acid was the VFA with highest concentration in OFMSW, BW+SS, and SS reactor and it shows that aceticlastic methanogenesis was the rate limiting step in those reactors. In BW reactor acetic acid and propionic acid were the main VFAs.

In all of the reactors concentration of propionic acid was lower than 900 mg/l which is considered as inhibitory concentration of propionic acid for methanogens (Wang et al. 2009). There were two relatively high concentration of butyric acid in BW+SS reactor (R2) and BW reactor (R3) (R2 day 49: 561 mg/l, R3 day 49: 429 mg/l) but it was lower than 1800 mg/l which is reported as no inhibitory effect of butyric acid on methanogenesis (Wang et al. 2009).

pH was in normal range, and similar to other studies at mesophilic condition (Cavinato et al. 2013) and not fluctuating too much. Stable pH supports the fact that there was not much VFA accumulation. The highest concentration of TAN was for OFMSW reactor effluent (843 mg/l) which was lower than 1700-1800 mg/l which is considered as inhibitory concentration of TAN (Yenigün et al. 2013).

SCOD in all of the reactors was lower in comparison to similar studies. The highest SCOD for SS and OFMSW in this study (0.7 g/l and 1 g/l respectively) were lower than SCOD of 1.62 and 2.16 g/l reported for SS (mixture of primary and secondary sludge) and household OFMSW (Cabbai et al. 2013).The highest SCOD for co-digestion in this study (1.4 g/l) was lower than SCOD of 6.72 g/l reported for co-digestion of FW and SS (Zhu et al. 2011). The highest SCOD for BW in this study was 1.8 g/l which was lower than SCOD of 46 g/l reported for the similar material by Gallert et al. (1997).

In the batch experiment BW had the highest methane yield (603 L CH_4/kg VS). OFMSW had the second highest methane yield (534 L CH_4/kg VS) and close to BW. SS had the lowest methane yield (369 L CH_4/kg VS). The reason for higher methane yield of BW could be positive effect of source separation on methane production. A comparison of methane yield of batch experiment in this study and previous studies are shown in Table 13.

Table 13. *Comparison of batch assay methane yields in this study and previous studies*

	Methane yield in this study (L CH_4/kg VS)	Methane yield in previous studies (L CH_4/kg VS)	Reference
Source-separated biowaste	603	527	Jokela et al. (2002)
OFMSW	534	525 353	Lissesns et al. (2004) El-Mashad et al. (2010)
Sewage sludge	369	248.8	Cabbai et al. (2013)

Methane yield of batch experiment for BW and SS in this study were higher than methane yield of 527 L CH_4/kg VS reported for source-separated BW (Jokela et al. 2002) and methane yield of 248.8 L CH_4/kg VS reported for SS (Cabbai et al. 2013). Methane yield of batch experiment for OFMSW in this study was similar or higher than previous studies. For example, Lissesns et al. (2004) reported methane yield of OFMSW as 525 L CH_4/kg VS and El-Mashad et al. (2010) reported methane yield of OFMSW as 353 L CH_4/kg VS at 35 °C.

9. CONCLUSION

In this study AD of mechanically treated OFMSW, source-separated BW, mixed SS and co-digestion of BW with SS were studied. AD of OFMSW, and BW were feasible in mesophilic process with OLR 1 to 2 kg VS/m^3 d and average methane yield of 386 and 385 L CH_4/kg VS respectively. AD of SS was done in mesophilic process with OLR of 0.3 to 1.1 kg VS/m^3 d resulting in average methane yield of 198 L CH_4/kg VS. BW and OFMSW had much higher (around 2 times) methane production and methane yield than SS. Higher methane yield of BW and OFMSW in comparison to SS can be related to their higher VS/TS ratio.

Co-digestion of BW and SS was shown to be feasible in mesophilic process with OLR 1 to 2 kg VS/m^3 d. The average methane yield was 318 L CH_4/kg VS which shows 61% increase in methane yield in comparison to mono-digestion of SS. Co-digestion of SS had the highest VS removal (85%) in four studied reactors. It can be considered as a positive effect of co-digestion. SS reactor had the lowest VS removal (36%), which can be a sign of poor performance of the reactor but usually reactors containing SS have low VS removal.

Process was stable after increasing OLR from 1 to 2 kg VS/m^3 d. There was not VFAs accumulation, and all of the VFAs concentrations were lower than inhibitory concentrations, which supports the fact that pH was in normal range (7-7.2) and there was not any need for pH adjustment. Low OLRs and long HRTs at the beginning of the experiment is important for a successful process, because in this way microorganisms will have time to adapt well for the feedstock.

A decrease in methane yield of OFMSW, BW and co-digestion of BW and SS after increasing OLR from 1 to 2 kg VS/m^3 d shows an optimal OLR for these digestion lower than 2 kg VS/m^3 d. Thus, optimization of OLR is an important factor, as higher OLR might result in lower methane yield. Another reason could be the short period of experiment. As in higher OLR the biogas production is slower, if the experiment was longer it might result in higher methane yield.

In the batch experiment methane yield of source-separated BW (603 L CH_4/kg VS) was higher than methane yield of mechanically treated OFMSW (534 L CH_4/kg VS). It can be considered as a positive effect of source separation on methane yield of organic solid waste in comparison to mechanical treatment. SS had the lowest methane yield in batch experiment (369 L CH_4/kg VS) which can be connected to its lower VS/TS ratio.

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